

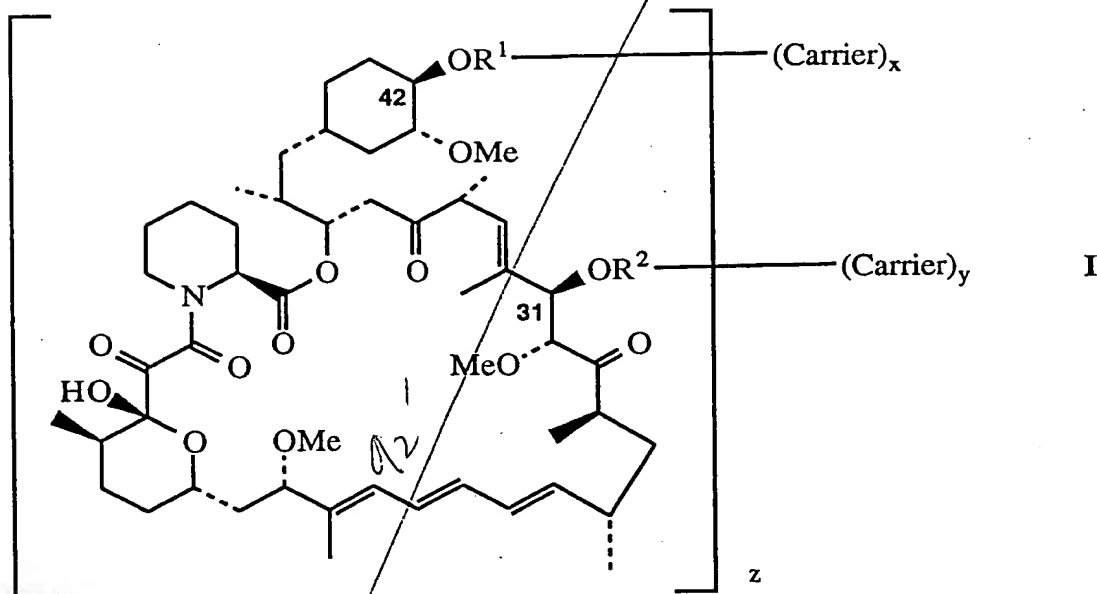
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CLAIMS

What is claimed is:

1. A rapamycin conjugate of formula I, having the structure

5



wherein R¹ and R² are each, independently, hydrogen or -(R³-L-R⁴)_a ;

L is a linking group;

- 10 R³ is selected from the group consisting of carbonyl, -S(O)- , -S(O)₂ , -P(O)₂- , -P(O)(CH₃)-, -C(S)-, and -CH₂C(O)- ;

R⁴ is a selected from the group consisting of carbonyl, -NH- , -S- , -CH₂- , and -O- ;

a = 1 - 5;

x = 0 - 1;

- 15 y = 0 - 1;

z is from about 1 to about 120;

and Carrier is immunogenic carrier material, detector carrier material, or a solid matrix, or a salt thereof with the proviso that R¹ and R² are both not hydrogen; and further provided that when a is greater than 1, each L group can be the same or different; and still further provided that x is 0 if R¹ is hydrogen and y is 0 if R² is hydrogen, and if x and y are both 1, the Carrier moiety is the same in both cases.

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2. An antibody, capable of specifically binding with rapamycin prepared against a conjugate of claim 1.

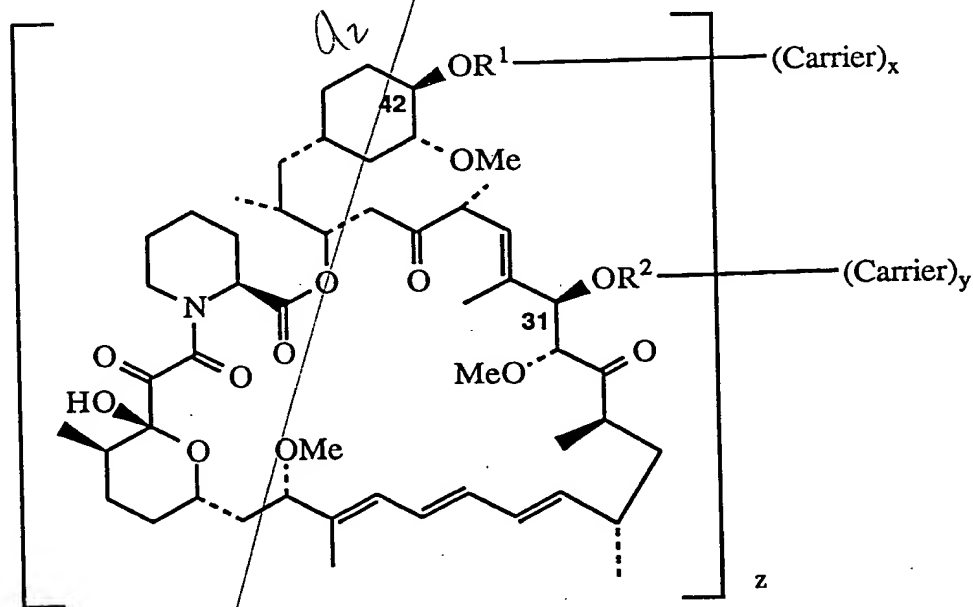
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3. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises employing an antibody prepared against a conjugate of claim 1.

4. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises using a conjugate of claim 1 as a detector molecule.

5. A test kit for measuring levels of rapamycin or a derivative thereof comprising a rapamycin conjugate of claim 1 bound to a solid support and an antibody capable of specifically binding to rapamycin.

6. A rapamycin conjugate of formula II, having the structure



R¹ and R² are each, independently, hydrogen or -R³-L-R⁴-;

L is -A-(CR⁵R⁶)ᵇ[B-(CR⁷R⁸)ᵈ]ᶜ-

A is -CH₂- or -NR⁹-;

20 B is -O-, -NR⁹-, -S-, -S(O)-, or -S(O)₂-;

R³ is selected from the group consisting of carbonyl, -S(O)-, -S(O)₂, -P(O)₂-,
-P(O)(CH₃)-, -C(S)-, and -CH₂C(O)-;

R⁴ is selected from the group consisting of carbonyl, -NH-, -S-, -CH₂-, and -O-;

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5 R⁵, R⁶, R⁷, and R⁸ are each, independently, hydrogen, alkyl of 1-6 carbon atoms, alkenyl of 2-7 carbon atoms, alkynyl of 2-7 carbon atoms, halo, hydroxy, trifluoromethyl, arylalkyl of 7-10 carbon atoms, aminoalkyl of 1-6 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, alkoxy of 1-6 carbon atoms, carbalkoxy of 2-7 carbon atoms, cyano, amino, -CO₂H, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or -CO₂H;

R⁹ is hydrogen, alkyl of 1-6 carbon atoms, or aralkyl of 7-10 carbon atoms;

10 b = 0-10;

d = 0-10;

e = 0-2;

x = 0 - 1;

y = 0 - 1;

15 z is from about 1 to about 120;

and Carrier is immunogenic carrier material, detector carrier material, or a solid matrix, or a salt thereof with the proviso that R¹ and R² are both not hydrogen; and further provided that when b is greater than 1, each of the CR⁵R⁶ groups can be the same or different, and when d is greater than 1, each of the CR⁷R⁸ groups can be the same or different; and still further provided that x is 0 if R¹ is hydrogen and y is 0 if R² is hydrogen, and if x and y are both 1, the Carrier moiety is the same in both cases.

25 7. The conjugate of claim 6, which is rapamycin 42-ester with succinic acid conjugate with keyhole limpet hemocyanin.

8. The conjugate of claim 6, which is rapamycin 42-ester with succinic acid conjugate with ovalbumin.

30 9. The conjugate of claim 6, which is rapamycin 42-ester with succinic acid conjugate with horseradish peroxidase.

10. The conjugate of claim 6, which is rapamycin 31,42-diester with glutaric acid conjugate with keyhole limpet hemocyanin.

35 11. The conjugate of claim 6, which is rapamycin 31,42-diester with glutaric acid conjugate with horseradish peroxidase.

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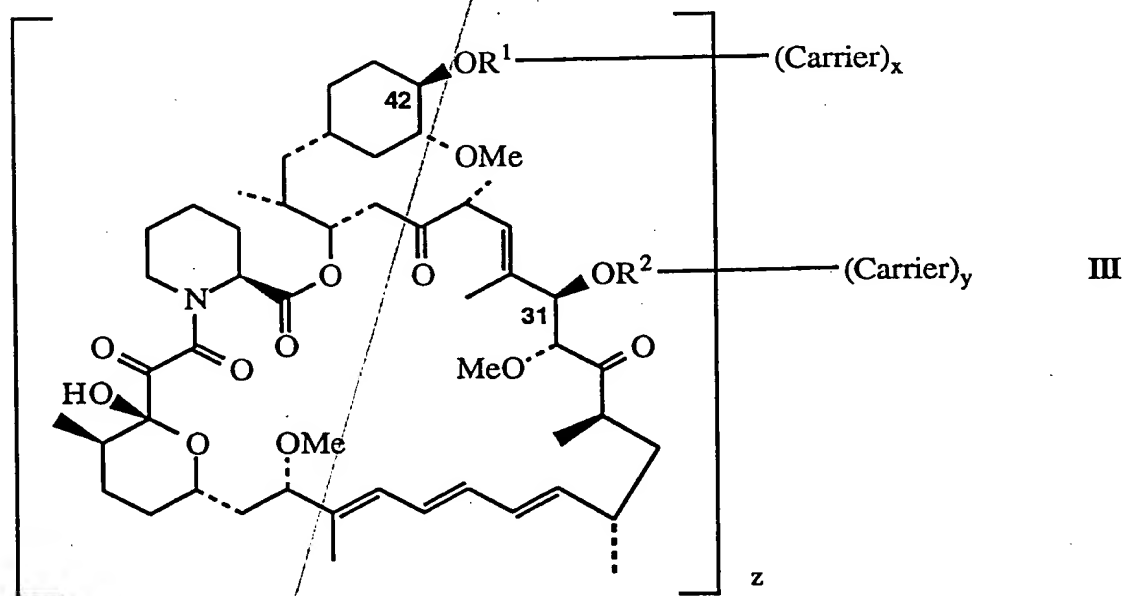
12. An antibody, capable of specifically binding with rapamycin prepared against a conjugate of claim 6.

5 13. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises employing an antibody prepared against a conjugate of claim 6.

10 14. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises using a conjugate of claim 6 as a detector molecule.

15 15. A test kit for measuring levels of rapamycin or a derivative thereof comprising a rapamycin conjugate of claim 6 bound to a solid support and an antibody capable of specifically binding to rapamycin.

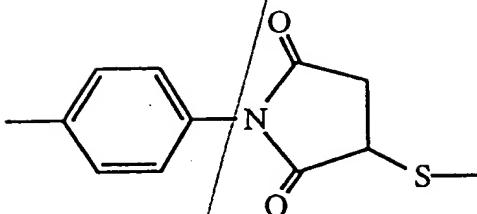
16. A rapamycin conjugate of formula III, having the structure



20 R^1 and R^2 are each, independently, hydrogen or $-(R^3-L^1-R^4)_f-(R^{10}-L^2-R^{11})_g$ -Carrier;
 L^1 is $-(CH_2)_h-CHR^{12}-(CH_2)_j-$;
 L^2 is $-(CH_2)_k-D-(CH_2)_m-E-$;

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D is $-\text{CH}_2-$, $-\text{S}-$, or



E is $-\text{CH}_2-$ or $-\text{C}(=\text{NH}_2^+\text{Cl}^-)-$;

R^3 and R^{10} are each, independently, selected from the group consisting of carbonyl, $-\text{S}(\text{O})-$, $-\text{S}(\text{O})_2-$, $-\text{P}(\text{O})_2-$, $-\text{P}(\text{O})(\text{CH}_3)-$, $-\text{C}(\text{S})-$, and $-\text{CH}_2\text{C}(\text{O})-$;

5 R^4 and R^{11} are each, independently, selected from the group consisting of carbonyl, $-\text{NH}-$, $-\text{S}-$, $-\text{CH}_2-$, and $-\text{O}-$;

10 R^{12} is hydrogen, alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, alkenyl of 2-7 carbon atoms, alkynyl of 2-7 carbon atoms, $-(\text{CH}_2)_n\text{CO}_2\text{R}^{13}$, $-(\text{CH}_2)_p\text{NR}^{14}\text{R}^{15}$, carbamylalkyl of 2-3 carbon atoms, aminoalkyl of 1-4 carbon atoms, hydroxyalkyl of 1-4 carbon atoms, guanylalkyl of 2-4 carbon atoms, mercaptoalkyl of 1-4 carbon atoms, alkylthioalkyl of 2-6 carbon atoms, indolylmethyl, hydroxyphenylmethyl, imidazoymethyl, halo, trifluoromethyl, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or $-\text{CO}_2\text{H}$;

15 R^{14} , and R^{15} are each, independently, hydrogen, alkyl of 1-6 carbon atoms, or arylalkyl of 7-10 carbon atoms;

20 R^{13} is hydrogen, alkyl of 1-6 carbon atoms, arylalkyl of 7-10 carbon atoms, alkenyl of 2-7 carbon atoms, alkynyl of 2-7 carbon atoms, or phenyl which is optionally mono-, di-, or tri-substituted with a substituent selected from alkyl of 1-6 carbon atoms, alkoxy of 1-6 carbon atoms, hydroxy, cyano, halo, nitro, carbalkoxy of 2-7 carbon atoms, trifluoromethyl, amino, or $-\text{CO}_2\text{H}$;

$f = 0-3$;

25 $g = 0-1$;

$j = 0-10$;

$k = 0-10$;

$m = 0-10$;

$n = 0-6$;

30 $p = 0-6$;

$x = 0-1$;

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$y = 0 - 1$;

z is from about 1 to about 120;

and Carrier is immunogenic carrier material, detector carrier material, or a solid matrix, or a salt thereof with the proviso that R^1 and R^2 are both not hydrogen; and further provided that f and g are both not 0 and when f is greater than 1, each of the $-(R^3-L^1-R^4)-$ moieties can be the same or different; and still further provided that x is 0 if R^1 is hydrogen and y is 0 if R^2 is hydrogen; and if x and y are both 1, the Carrier moiety is the same in both cases.

10 17 The conjugate of claim 16, which is rapamycin 42-ester with 3-[3-(4-aminobutylthio)succinimidyl]phenacylglycine conjugate with horseradish peroxidase.

18. The conjugate of claim 16, which is rapamycin 42 ester with (N-(3-carboxyphenyl)-3-thiosuccinimidyl)-glycine conjugate with horseradish peroxidase.

15 19. An antibody, capable of specifically binding with rapamycin prepared against a conjugate of claim 16.

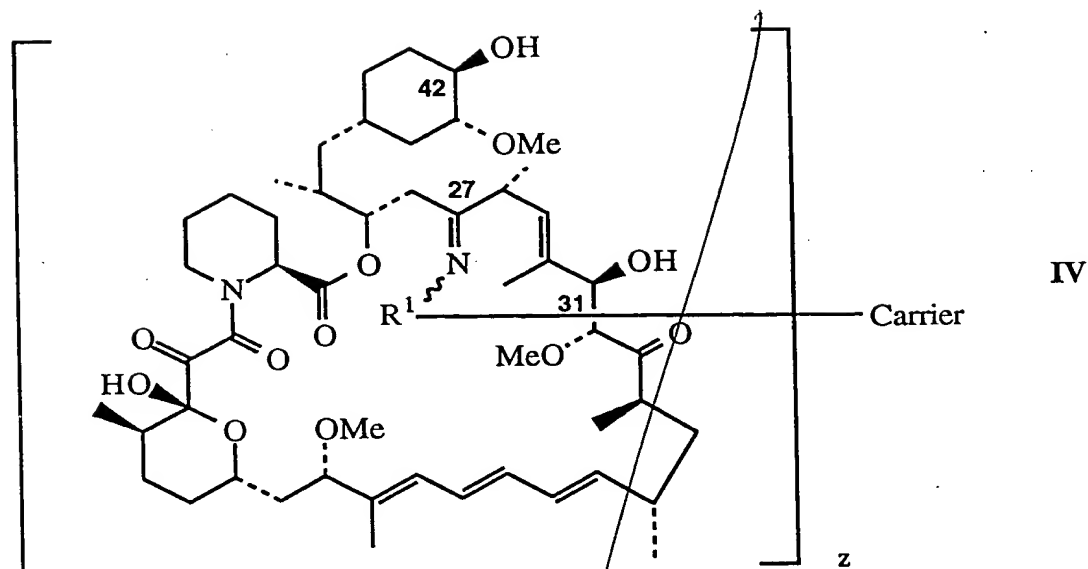
20 20. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises employing an antibody prepared against a conjugate of claim 16.

21. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises using a conjugate of claim 16 as a detector molecule.

22. A test kit for measuring levels of rapamycin or a derivative thereof comprising a rapamycin conjugate of claim 16 bound to a solid support and an antibody capable of specifically binding to rapamycin.

30 23. A rapamycin conjugate of formula IV, having the structure

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wherein R^1 is $-OCH_2(CH_2)_qR^4-$;

R^4 is selected from the group consisting of carbonyl, $-NH-$, $-S-$, $-CH_2-$, and $-O-$;
 $q = 0 - 6$;

5 z is from about 1 to about 120;

and Carrier is immunogenic carrier material, detector carrier material, or a solid matrix, or a salt thereof.

24. An antibody, capable of specifically binding with rapamycin prepared against a conjugate of claim 23.

25. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises employing an antibody prepared against a conjugate of claim 23.

26. In an immunoassay method for determining levels of rapamycin or a derivative thereof, the improvement comprises using a conjugate of claim 23 as a detector molecule.

27. A test kit for measuring levels of rapamycin or a derivative thereof comprising a rapamycin conjugate of claim 23 bound to a solid support and an antibody capable of specifically binding to rapamycin.

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28. A monoclonal antibody specifically binding to rapamycin which is designated as RAP-42-OVAF₂#1MoAb.
29. A hybridoma cell line capable of producing rapamycin specific antibodies which is designated as RAP-42-OVAF₂#1hc-.
30. A test kit for measuring levels of rapamycin or a derivative thereof, comprising a rapamycin specific antibody bound to a solid support.
31. A test kit for measuring levels of rapamycin or a derivative thereof, comprising a molecule bound to a solid support capable of capturing a rapamycin specific antibody.
32. The test kit according to claim 31 wherein the bound molecule is goat anti-mouse antibody.

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